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THE REACTIONS OF *PARAMECIUM* TO SOLUTIONS OF KNOWN HYDROGEN ION CONCENTRATION.[1]

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INTRODUCTION.

The reactions of unicellular organisms to acids in dilute solution has been a subject for research for many years. Pfeffer in 1888 observed the positive reaction of spermatozoa to malic acid. Jennings ('97, '99*a*, '99*c*, '00*a*, '00*b*, '00*c*, '02, '06) described the attracting action of dilute acids for *Paramecium* and other protozoa. He expressed quantitative results in terms of fractional normality of acids rather than concentration of hydrogen ions. Garrey ('00), a student of Loeb, studying the reactions of *Chilomonas* to acids recognized the necessity for measuring the results in terms of hydrogen ion concentration but was able to do it only relatively rather than absolutely. Garrey and Jennings achieved results at variance and became involved in a lively controversy over the interpretations—a part of the larger Loeb-Jennings controversy. Greeley ('04) obtained positive reactions to dilute acid solutions with *Paramecia* taken from acid cultures. He states that he did not obtain positive reactions with *Paramecia* taken from alkaline cultures.

This field seems to have been abandoned by later workers. With the discovery of methods for determining the true hydrogen ion concentration of fluids and materials in recent years numerous investigations have been made concerning the part of acidity and alkalinity in biological processes, but none of these investigations have been along the lines followed by Jennings, Garrey

[1] The writer is greatly indebted to Dr. W. C. Allee for suggesting this investigation and for facilities and advice afforded in connection with it and to Mr. M. R. Garner for his coöperation in the initial experiments.

and Greeley. Later workers (Fine, '12, Collett, '19, Kostir, '21, Bodine, '21, Saunders, '24, Hopkins, '26, Pruthi, '27, Beers, '27, Eddy, '28, Darby, '29) have been more concerned with the tolerance of organisms to acids, the toxicity of acids in the media, the changes in natural acidity in protozoan cultures, and the effects of hydrogen ion concentration on different life processes.

No account of a repetition of the experiments performed by Jennings, Garrey and Greeley using modern methods of determining hydrogen ion concentration, as worked out by Sorensen and Clark and Lubs, has been found in the literature. The series of experiments described in this paper was undertaken to determine the reactions of *Paramecia* to acid solutions of known hydrogen ion concentration. The experiments were started during the summer of 1927 [1] in Whitman Laboratory and were continued there during the summers of 1928–29. During the winters of 1927–28 and 1928–29 the work was carried on at Wabash College, Crawfordsville, Indiana.

Methods and Materials.

A preliminary study was made to find satisfactory methods for carrying on the main series of experiments. Pfeffer (fide Loeb, '18) introduced the open end of a capillary tube sealed at the other end and filled with acid solution, into a drop of organisms. Loeb ('18) observes that Pfeffer's method of testing chemotropism is the best. Barratt (fide Loeb, '18) concluded, using Pfeffer's capillary tubes as described above, that *Paramecia* were indifferent to acid solutions. Barratt's experiments were repeated using HCl (pH 5.2) in capillary tubes and like results were obtained. When slightly larger tubes were used the organisms entered the acid in about ten times the numbers that entered the control. Ordinary pipettes were used with the result that seventy-five animals entered the acid while one entered the control. From these results it seemed evident that in the case of very small tubes it is almost impossible for a *Paramecium* swimming spirally to enter without striking the edge of the opening. And so Pfeffer's capillary tubes were not considered

[1] Mr. M. R. Garner and the present writer worked together on the first set of experiments to be described.

practical in these experiments. Jennings ('06) placed a cover slip, slightly elevated with glass rods, on a slide; introduced a drop of medium containing organisms on one side and acid solution on the other by means of a capillary pipette, or, merely placed a bit of acid at the edge of a cover slip under which the organisms lay. Garrey ('00) used a hollow cell for holding the organisms and introduced the acid through a small opening on one side. In addition to these methods two others were tried. (1) An acid gradient was made in a long tube (24 inches); the organisms were introduced at the end of the gradient corresponding in H-ion concentration to their own medium, and (2) a "double drop" method was devised in which a drop of medium containing *Paramecia* and a drop of acid solution were placed side by side on a slide and the two drops were connected by leading the liquids toward each other in a narrow line with the point of a needle. The former of these methods was discarded as being utterly impractical and the latter was chosen for use in preference to the older methods for a number of reasons. (1) Less elaborate apparatus was required. (2) The experiments were set up and carried on more quickly. (3) A more gradual gradient could be established than with the older methods.

Trays were made for holding the slides during the experiments. These trays, made by Mr. Carson in the shop at Whitman Laboratory, were fifteen inches in length, four inches in width and two and a half inches in depth with grooves on the sides to hold the slides in place. It was found that strips of black paper placed in the bottom of the trays aided greatly in making the counts at the beginning and end of each experiment.

The colorimetric method of determining hydrogen ion concentration was used except as noted. The standard tubes and indicators used were purchased from the La Motte Chemical Products Company or the Hynson, Westcott and Dunning Company, both of Baltimore, Md. The standard tubes covered a range from pH 3.0 to pH 8.4. The indicators were brom phenol blue, brom cresol green, methyl red, brom cresol purple and phenol red. The indicators, with the exception of methyl red, were made up in aqueous solutions. The exception was made up in an alcoholic solution as it is only slightly soluble in water, but the quantity of alcohol introduced into a solution when this indicator was used was far below what Jennings found to elicit any sort of response. Both methyl red and brom cresol green were used in determining pH 5.0. Neither seemed to give very accurate and reliable results because the color change at this point is not very marked in the case of both. Hyman ('25) has noted this same difficulty with brom cresol green. Due to this difficulty it seems possible to explain some of the inconsistencies in the data at H-ion concentrations of pH 5.0.

Preliminary experiments were made to determine the degree of diffusion between the two drops. The usual result is illustrated in Fig. 1. Complete diffusion required about an hour while the

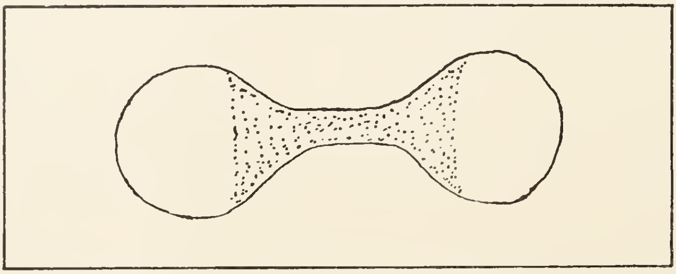

FIG. 1.

experiments usually ran about half an hour. The culture drops start drying first so any reading taken after half an hour would likely be affected by the drying in the culture drop. It was found that if the slides were too clean the drops spread rather badly. This could usually be remedied by rubbing one's hand over the slide to coat it with a film of oil. Experiments were carried on in duplicate, *i.e.* two slides bore the same drop combination. Experiments rendered unsatisfactory on account of the drops spreading or for other reasons were discarded. It was determined that it made no difference if the indicator was included in the acid solution.

Hay infusions were prepared by boiling 5 grams of timothy hay in 500 cc. of water for about five minutes. An additional 500 cc. of unboiled water was added to the infusion and after it had stood for a few days it was seeded with *Paramecia*. *Paramecium caudatum* as described by Wenrich ('28) were used.

The original seed was obtained from Dr. Peterson in Whitman Laboratory and all additional cultures were seeded from this strain. The cultures were not used until they were two or three weeks old. A new culture was prepared each week so that a number of cultures were on hand throughout the experiments. At first the cultures were kept in 1000 cc. Erlenmeyer flasks. It was found that the H-ion concentration of such cultures remained high for a long time. The cultures for practically all of the experiments were kept in wide-mouthed bottles and finger bowls. According to Jennings ('06), the fact that *Paramecia* collect in cultures in clumps where carbon dioxide is present often causes misleading results, so in all of the experiments the *Paramecia* were stirred and aërated to eliminate this factor.

The following acids were used: hydrochloric, nitric, sulphuric, carbonic, formic, acetic, citric, picric, pyrogallic, and tannic. Carbonic acid was prepared by bubbling CO_2 into water until the desired H-ion concentration was obtained. As the CO_2 soon escapes into the air and thus changes the H-ion concentration, it seemed necessary to find a way of covering the drops when carbonic acid was used. This was done by cementing glass strips on the edges of ordinary slides. The tray holding these "chamber slides" was placed in a large covered pan and CO_2 was allowed to escape into the pan. Then the drops were placed in the "chamber slides" and connected as quickly as possible. As soon as the drops were connected each "chamber slide" was covered with an ordinary slide. Indicators showed that the change in H-ion concentration from the time the drop was placed on the slide until it was covered was not great.

The following different kinds of water were used in making up the acid solutions: ordinary Chicago tap water, Hull[1] well water, Whitman[2] well water, Crawfordsville (Ind.) tap water, distilled water, Chicago tap water made carbonate-free, Whitman well water made carbonate-free and Hull well water made carbonate-free. The carbonate-free water was made according to the method used by Hyman ('25). Two cc. of concentrated

[1] From Hull Zoölogical Laboratory.

[2] From Whitman Experimental Laboratory.

hydrochloric acid were used to eight liters of water in a large bottle. Air from the compressed air system was passed through the water in the bottle for at least 24 hours. Analyses of the Whitman well water and the Hull well water will appear later in the paper.

At first there was a question as to what constituted a positive reaction. The constant moving about of the *Paramecia* made it appear that some of the crossing over from one drop to another must be attributed to random movements on the part of the *Paramecia*. It was thought that the absence of *Paramecia* from one drop might be a factor in causing the crossing over. A set-up was made to test this idea. Some culture liquid from a culture with a concentration of pH 7.7 was centrifuged. The culture fluid, free from *Paramecia*, was drawn off and used in an experiment in the same way that acidified water had been used—a drop of the culture fluid free from *Paramecia* was joined to a drop of culture containing *Paramecia*. There was no change in the H-ion concentration from centrifuging. Table I gives the result of this experiment. At the time of reading which was that used in other experiments about equal numbers were found in each drop. On the basis of these results, reactions of over 55 per cent were considered as positive reactions. This indicates that the absence of *Paramecia* from one of the drops is not a factor in causing the positive reactions.

Table I.

Showing Amount of Random Movement on Part of *Paramecia*.

	pH 7.7 (Centrifuged Culture liquid)					
	45	55	50	51	46	48
	46	46	56	60	51	73
	53	58	41	49	56	52
	36	61	40	40	53	43
	56	56				26/1326
Ave. % reaction						51

A number of experiments were undertaken during the Christmas holidays (1928–29) but not many were successful. The temperature of the laboratory during this time ranged from 15° C.

to 21° C. The only experiments which gave any positive results were conducted when the temperature was 21° C. The experiments run when the temperature was lower than 21° C. gave no positive results. Jennings ('06) states that Mendelssohn found that the optimum temperature for *Paramecium* is between 24 and 28 degrees C. All of the experiments recorded here were conducted within a temperature range of 21 to 28 degrees C. No data were obtained as to the upper temperature limit for positive reactions.

Experiments.

(1) *To Determine pH Range in which Positive Reactions Occur.*

In the beginning several experiments were carried out using a pH range of 3.2 through 5.8. It became evident, after using several acids and repeating the experiments, that no positive reaction (it was considered that the *Paramecia* must enter the acid drop) could be obtained at a H-ion concentration greater than pH 5.0 with any of the acids used. Any H-ion concentration of pH 4.8 or lower proved to be toxic to *Paramecium*. So with the lower limit for positive reactions established, the next problem was to determine the reactions of *Paramecium* to a range of H-ion concentrations of pH 4.8–5.8. The following table shows the results obtained with hydrochloric, sulphuric and nitric acids in Chicago tap water.

Section *A* of this table shows the results of six set-ups. Two slides with the same H-ion concentration were used in each set-up, as the table shows. The numbers in the different columns represent the percentage reaction on each slide from which the mean average reaction was obtained. The number of *Paramecia* on each slide averaged about fifty. This was true for all of the experiments. According to the criterion established above Table II. shows positive reactions from pH 5.0 through pH 5.8 in the three inorganic acids used. There seemed to be no significant difference in the reactions with the different acids. The H-ion concentration of the cultures used in these experiments ranged from pH 7.4 to pH 8.0. Controls (in duplicate) were run in each set-up by joining a drop of culture with a drop of unaltered tap water. No positive reaction occurred in any of the controls. The high percentage reaction (81 per cent.) at

Table II.

Showing the Percentage Reactions of *Paramecia* to Hydrogen Ion Concentrations of pH 4.8–5.8 Made with Hydrochloric, Sulphuric and Nitric Acids in Chicago Tap Water.

(Data in section *A* obtained in conjunction with Mr. M. R. Garner.)

A (1927).

	pH 4.8	5.0	5.2	5.4	5.6	5.8
	10	73	96	84	80	22
Hydrochloric	12	84	70	73	88	25
	25	18	70	90	67	78
Hydrochloric	10	27	92	80	50	79
	23	23	95	70	72	76
Sulphuric	4	42	89	71	70	69
	41	56	59	54	43	74
Sulphuric	58	87	74	55	39	18
	60	68	87	55	56	61
Nitric	45	88	65	65	60	51
	22	65	82	75	52	50
Nitric	58	56	92	59	83	58
	12/368	12/686	12/971	12/829	12/760	12/661
Ave. % reactions	30	57	81	69	63	55

B (1928).

	pH 4.8	5.0	5.2	5.4	5.6	5.8	7.8
	35	73	53	67	80	91	
Hydrochloric	70	83	54	59	89	94	40
	23	41	43	47	91	70	
Hydrochloric	50	47	77	37	97	78	6
	44	65	54	80	23	30	
Hydrochloric	20	63	95	30	56	63	9
	36	53	81	70	74	71	
Hydrochloric	52	76	92	70	100	97	26
	44	65	92	54	72	98	
Sulphuric	60	40	80	51	78	75	41
	41	72	67	97	67	67	
Sulphuric	58	50	83	54	63	72	9
	9	70	74	77	60	69	
Sulphuric	34	74	70	54	50	85	30
	15	90	85	72	83	64	
Sulphuric	17	80	77	72	77	61	21
	13	70	67	82	70	60	
Nitric	22	81	67	82	52	87	34
	18	50	73	62	80	77	
Nitric	20	82	70	71	80	65	0
	28	82	50	74	88	70	
Nitric	29	80	60	81	80	68	8
	22/738	22/1487	22/1564	22/1463	22/1610	22/1611	11/224
Ave. % reactions	33	67	71	66	73	73	20

pH 5.2 seemed to be a possible optimum but later experiments did not indicate such a marked reaction to any one H-ion concentration. In all of the experiments the temperature of the acid solutions was maintained the same as the temperature of the culture used.

The results in Section *B* are very similar to those listed in Section *A*. One difference seems to be that there is no marked optimum in the reaction given at any pH as was the case in the reactions to a concentration of pH 5.2 in Section *A*. The figures in the column under pH 7.8 show the results in the controls which were like the controls used in the first series of experiments. The cultures used ranged in H-ion concentration from pH 7.4 through pH 7.8. Another difference is evident in that the percentage of reactions at pH 5.8 in the second series is no lower than at any other pH in the range used. Thus it became necessary to extend the range of experimentation. A greater range had not been used in the first series because time would not permit.

Several organic acids were used over the same pH range. Acetic, citric and formic acids elicited responses somewhat comparable to those of the inorganic acids as shown in Table II. Pyrogallic and tannic acids proved to have such a high degree of toxicity for the animals that reactions within the pH range studied were impossible—death resulting on contact with the weakest part of the diffusion area. This high degree of toxicity of tannic acid may offer an explanation of the absence of Protozoa from bog waters. Picric acid was used several times with inconsistent results. The strong color of the acid solution prevented accurate pH readings. It also seemed that here, as with pyrogallic and tannic acids, there is some other factor besides the H-ion concentration which affects the reactions of *Paramecia*.

Later in the summer of 1927 cultures with higher H-ion concentrations (pH 6.8, pH 7.0 and pH 7.2) were used and no positive results were obtained at the above ranges. Several times throughout the period of experimentation cultures with these concentrations were used with the same results—no positive reactions. When the H-ion concentration of the cultures fell, positive results were obtained.

Table III. shows that positive reactions can be obtained in a pH range of 6.0–6.8, which, taken with the results of the two other series, means that *Paramecia* from alkaline cultures react positively throughout a pH range of 5.0–6.8. No positive response was obtained in the controls at pH 7.8.

Table III.

Showing the Percentage Reactions of *Paramecia* to Hydrogen Ion Concentrations of pH 6.0–6.8 Made with Hydrochloric, Sulphuric and Nitric Acids in Chicago Tap Water.

	pH 6.0	6.2	6.4	6.6	6.8	7.8
	44	78	88	57	79	
Hydrochloric	62	66	66	65	54	38
	54	84	81	61	76	
Hydrochloric	83	85	55	70	77	2
	40	40	62	80	63	
Sulphuric	53	40	70	58	80	0
	51	48	51	58	62	
Sulphuric	33	51	36	52	57	38
	89	77	81	77	57	
Nitric	80	43	73	70	60	21
	46	48	46	43	56	
Nitric	57	49	54	50	46	30
	46	81	64	59	69	
Nitric	76	75	54	65	76	0
	14/814	14/865	14/881	14/865	14/914	7/129
Ave. % reactions	58	62	63	62	65	18

A series of experiments were run using Hull well water. The following table gives the results when unaltered Hull well water was acidified with nitric, hydrochloric and sulphuric acids.

The cultures used in Table IV. had a H-ion concentration of pH 7.8. Unaltered Hull well water, pH 7.8, was used in the controls. These data show that *Paramecia* from cultures with a low H-ion concentration (pH 7.8) react about the same way to acidified Hull well water as they react to acidified Chicago tap water.

All the data seem to point rather conclusively to the fact that *Paramecium caudatum* as reared in these cultures do not react positively to H-ion concentrations greater than pH 5.0. As one of the objects of this problem was to determine the pH range for positive reactions, it seemed necessary at this point to run more experiments attempting to determine the upper limit for positive responses. Crawfordsville (Ind.) tap water, coming from a number of surface springs, was used. This water always had a H-ion concentration of pH 7.4. Table V. gives the results of a series of experiments using unaltered Crawfordsville tap water, acidified with hydrochloric, sulphuric and nitric acids, over a pH range of 5.0 to 7.2. The unaltered tap water, pH 7.4, was used in the series as a control. The cultures used in this series had H-ion concentrations of pH 7.6 to pH 7.8.

Table IV.

Showing the Percentage Reactions of *Paramecia* to Hydrogen Ion Concentrations of pH 4.8–5.8 Made with Hydrochloric, Sulphuric and Nitric Acids in Hull Well Water.

	pH 4.8	5.0	5.2	5.4	5.6	5.8	7.8
	9	72	90	85	79	79	
Hydrochloric........	23	80	79	70	71	89	47
	20	36	60	90	93	70	
Sulphuric...........	15	20	50	83	79	65	43
	10	40	70	84	80	79	
Nitric..............	17	30	81	89	89	76	26
	6/94	6/278	6/430	6/501	6/489	6/458	3/116
Ave. % reactions....	16	46	72	83	81	76	38

It will be noticed by examining Table V. that positive reactions were obtained throughout the entire range used—pH 5.0–7.2. And not only were positive reactions obtained throughout this range, pH 5.0–7.2, in which the H-ion concentration was produced by adding acid to the water, but positive reactions were also obtained in the controls, unaltered tap water, with a H-ion concentration of pH 7.4. If all of the preceding tables are examined it will be found that in the other controls the H-ion concentration in each case was the same as, or lower than, the H-ion concentration of the culture used. In this case the H-ion concentration of the control used was higher than the hydrogen ion concentration of the cultures used. This pointed to another set of experiments using water with a H-ion concentration the same as the culture used. All of the controls of the preceding experiments indicate that *Paramecium* will not react positively to a solution with a H-ion concentration the same as, or lower than, the H-ion concentration of the culture. Indicators showed

Table V.

Showing the Percentage Reactions of *Paramecium* to Hydrogen Ion Concentrations at pH 5.0–7.2 Made with Hydrochloric, Sulphuric and Nitric Acids in Crawfordsville Tap Water.

	pH 5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4
HCl.	44	74	83	67	87	80	75	71	74	48	79	64	73
	52	65	62	70	81	81	75	74	53	70	83	83	70
HCl.	43	68	63	86	82	81	84	70	87	74	74	91	59
	70	76	85	84	86	69	78	79	80	75	72	81	69
HCl.	84	75	88	78	92	69	80	72	69	72	89	75	78
	63	70	85	88	81	76	64	79	85	71	86	78	73
HCl.	51	57	72	76	81	80	65	69	67	78	73	73	76
	57	70	80	64	70	75	59	75	69	62	78	72	65
HCl.	54	67	80	100	86	78	86	88	85	84	89	88	58
	62	80	54	86	65	57	74	79	93	71	75	88	56
HCl.	33	55	71	83	74	72	83	56	65	82	73	84	69
	31	47	68	84	74	81	77	62	89	81	80	74	58
H_2SO_4.	27	43	78	81	77	92	79	89	83	84	79	89	63
	32	48	86	79	84	89	88	72	87	81	88	87	56
H_2SO_4.	62	60	81	76	77	79	80	68	77	80	80	83	82
	55	77	73	72	70	75	82	74	75	77	79	75	78
H_2SO_4.	30	53	62	65	74	69	75	60	50	54	49	57	61
	48	24	68	50	79	75	69	60	53	40	70	46	83
H_2SO_4.	24	92	86	91	91	92	100	89	82	81	86	83	77
	25	91	84	87	89	91	87	89	66	80	77	86	83
H_2SO_4.	26	41	32	67	84	50	62	52	40	31	60	84	83
	27	44	32	60	62	41	55	44	46	41	66	79	87
H_2SO_4.	45	47	75	60	75	60	71	87	72	70	76	50	61
	59	63	72	61	51	78	75	66	72	74	50	56	73
HNO_3.	64	83	90	90	76	79	94	35	75	88	70	79	84
	53	79	81	76	81	84	85	85	72	89	90	88	82
HNO_3.	41	53	61	53	79	70	77	84	88	81	51	53	79
	53	56	60	80	53	69	80	83	91	85	54	82	59
HNO_3.	26	57	60	58	71	44	40	75	55	53	70	76	33
	20	56	63	61	74	46	52	63	51	67	89	64	41
HNO_3.	59	46	81	80	71	69	73	79	63	74	86	67	76
	55	49	74	89	79	85	75	79	74	71	77	76	80
HNO_3.	56	43	60	93	85	76	41	66	64	82	50	65	64
	58	54	93	84	70	60	38	84	73	81	68	65	70
Ave. % reactions	34/1592	34/2063	34/2443	34/2574	34/2601	34/2470	34/2478	34/2455	34/2435	34/2432	34/2516	34/2541	34/2359
	47	61	72	76	76	72	72	72	71	71	74	74	69

that the H-ion concentration of the drops made from Crawfordsville tap water fell as soon as the drops were exposed to the air, so it was possible by aërating the water to lower the H-ion concentration to pH 7.8. Table VI. gives the results of the set of experiments using *Paramecia* from a culture with a concentration of pH 7.8 and Crawfordsville tap water aërated so that the concentration was pH 7.8. A control was used by testing the reaction of *Paramecia* to a concentration of pH 5.6 made by adding nitric acid to Crawfordsville tap water. Positive reactions were obtained in the controls but not in the drops where the H-ion concentration of the two drops was the same. This would seem to indicate the *Paramecia* from cultures with a low H-ion concentration react positively to any H-ion concentration higher than that of their culture to a pH of 5.0, which is the lower limit for positive reactions.

Table VI.

Showing the Percentage Reactions of *Paramecia* to a Hydrogen Ion Concentration of pH 7.8 Made by Aërating Crawfordsville Tap Water.

	5.6 (Nitric Acid).	7.8 (Aërated Crawfordsville Tap Water).				
	75	11	12	35	44	31
	82	4	18	40	28	41
	61	27	16	24	33	27
	78	38	27	24	36	11
	4/296	36	41			22/639
Ave. % reactions..	74					29

(2) To Determine Whether the Positive Reactions are Due to Carbon Dioxide or to Hydrogen Ions.

Jennings ('06) suggests that the positive reactions of *Paramecia* to dilute acids is closely connected with their natural tendency to collect in clumps when a large amount of carbon dioxide is present. Hyman ('25) found that "the depressing action of acids in natural waters is due chiefly or wholly to the carbon dioxide which they liberate from the carbonates of such waters." With these ideas in mind a series of experiments was run in an attempt to determine whether the *Paramecia* react to carbon dioxide or to the free hydrogen ions. Twelve sets (like the sets described in the preceding tables) were run from pH 4.8–6.8 with carbonate-free water made from Whitman well water using hydrochloric, sulphuric and nitric acids with no positive results. I was ready to conclude from these results that *Paramecia* react to carbon dioxide and not to free hydrogen ions when I realized that Whitman well water had not been used in any of the preceding experiments. So twelve more sets were run over the same range using Whitman well water as it comes from the tap and acidified with the same acids. These results were also negative. As a result the use of Whitman well water had to be discontinued. I can give no reason for the negative results. Analyses of Whitman well water, as well as of Hull well water with which positive results were obtained, were made but the differences were not great. No presence of B. coli was found in Whitman well water; so it seems that the unfavorable reactions to this water must be due to obscure chemical causes.

Some Hull well water was then made carbonate free in the manner already described. The carbonate-free water had a pH of 4.6. The pH was raised to 7.8 by adding NaOH. The water was then acidified. Table VII. shows the results of a series of experiments using carbonate-free Hull well water acidified with hydrochloric, sulphuric, and nitric acids.

Table VII.

Showing the Percentage Reactions of *Paramecia* to Hydrogen Ion Concentrations of pH 4.8–5.8 Made with Hydrochloric, Sulphuric and Nitric Acids in Carbonate-free Hull Well Water.

	pH 4.8	5.0	5.2	5.4	5.6	5.8	7.8
	48	67	53	55	89	51	7
Hydrochloric	40	40	78	70	87	57	34
	39	34	55	70	83	75	3
Sulphuric	60	65	54	92	81	77	3
	51	66	54	56	86	79	27
Nitric	30	60	54	75	94	73	23
	6/268	6/332	6/348	6/418	6/520	6/412	6/97
Ave. % reactions	45	55	58	69	86	69	16

The cultures used in the above experiments had *a* concentration of pH 7.6. Carbonate-free Hull well water with a sufficient amount of sodium hydroxide to make the pH 7.8 was used in the controls. This series of experiments indicated that the *Paramecia* were reacting to the free hydrogen ions and not to the carbon dioxide.

The reaction of *Paramecia* to Hull carbonate-free well water acidified with carbonic acid was tried. The carbonate-free water was lowered to pH 7.8 with sodium hydroxide. Carbon dioxide was then passed through the water until the desired acidity (pH 5.6) was obtained. It was found that carbonic acid elicited a response similar to that elicited by the three inorganic acids. The experiment was carried out in the manner described earlier in the paper. Table VIII. shows the results. The cultures used had H-ion concentrations of pH 7.7 and pH 7.8. Carbonate-free water with sodium hydroxide added to it giving a H-ion concentration of pH 7.8 was used in the controls.

Table VIII.

Showing the Percentage Reactions of *Paramecia* to a Hydrogen Ion Concentration of pH 5.6 Made with Carbonic Acid in Carbonate-free Hull Well Water.

	pH 5.6	7.8
	76	40
Carbonic acid	71	20
" "	94	25
" "	88	25
" "	76	0
" "	73	20
" "	75	45
" "	50	20
" "	73	11
" "	12	21
" "	44	37
" "	64	10
" "	89	4
" "	70	3
" "	78	20
" "	67	9
" "	83	11
" "	70	21
	18/1253	18/342
Ave. % reactions	70	19

Chicago tap-water was made carbonate-free in the same manner and used in a series of experiments. The Chicago carbonate-free tap-water tested pH 4.6 when it was made, and, as in the experiments with Hull carbonate-free well water, the H-ion concentration was lowered to pH 7.8 by adding sodium hydroxide. Table IX. gives the results of a series of experiments using Chicago carbonate-free tap-water. The cultures used tested pH 7.8. Carbonate-free water plus sodium hydroxide with a H-ion concentration of pH 7.8 was used in the controls. The results are like those obtained in the other experiments over this pH range except in the cases where Whitman well water was used. These results also indicate that the *Paramecia* react to free hydrogen ions and not to carbon dioxide.

Table IX.

Showing the Percentage Reactions of *Paramecia* to Hydrogen Ion Concentrations of pH 4.8–5.8 Made with Hydrochloric, Sulphuric and Nitric Acids in Carbonate-free Chicago Tap-Water.

	pH 4.8	5.0	5.2	5.4	5.6	5.8	7.8
	53	86	52	72	86	86	21
Hydrochloric	55	33	88	61	76	63	46
	46	63	74	40	83	65	21
Sulphuric	30	64	76	83	81	46	42
	42	44	61	85	86	66	22
Nitric	20	41	62	77	75	64	16
	6/246	6/331	6/413	6/418	6/487	6/390	6/168
Ave. % reactions	41	55	69	69	81	65	28

(3) *To Determine Reactions of Paramecia to Distilled Water.*

Two sets of experiments were run using distilled water. The distilled water tested pH 5.6 and the *Paramecia* reacted to it positively. However, in order to run a series of experiments over the pH range 4.8 to 5.8, the H-ion concentration of the distilled water was lowered to pH 7.8 by adding NaOH. The water was then acidified to obtain the desired H-ion concentrations. The cultures used had a H-ion concentration of pH 7.7–7.8. Distilled water plus NaOH with a H-ion concentration of pH 7.8 was used in the controls. Table X. gives the results of two sets of experiments using distilled water altered with NaOH. Positive results were obtained but these results differed from any of the other results obtained using inorganic acids in this respect; in all of the drops the *Paramecia* were dead, or at least becoming inactive, at the time of the reading.

Table X.

Showing the Percentage Reactions of *Paramecia* to Hydrogen Ion Concentrations of pH 4.8–5.8 Made with Hydrochloric and Nitric Acids in Distilled Water.

	pH 4.8	5.0	5.2	5.4	5.6	5.8	7.8
	13	67	65	56	61	100	10
Hydrochloric........	60	54	60	42	63	62	7
Nitric..............	25	82	77	63	75	54	27
	37	88	69	77	60	76	25
	4/135	4/291	4/261	4/238	4/259	4/292	4/69
Ave. % reactions....	34	73	65	60	65	73	17

(4) *To Determine pH at the End of the Experiments.*

It was evident that the H-ion concentration in the drops fell during the duration of the experiments. An attempt was made to determine the H-ion concentration at the end of the experiments. The sulphonphthalein indicators apparently are not harmful to *Paramecia* and do not affect their reactions when no greater concentration of the indicator is used than is necessary for making a pH reading in one of the tubes. Indicators were added to the drops used in the experiments. The results of many observations are given in Table XI. It is very difficult to make pH readings of the drops at the end of the experiment in this way. The density of the color in the drops is much less than the density of the color in the tube when the pH reading is determined. If enough of the indicator is added so that the density of the color in the drops is like that in the standard tubes, it is impossible to make accurate pH readings of the liquid to start with. So these observations recorded in Table XI. must be considered only as approximations. The writer is not convinced that the H-ion concentration at the end of the experiment is of very great importance because in many cases quite positive reactions occurred before the H-ion concentration had changed to any appreciable degree.

(5) *To Compare Determinations Made with Indicators and with Potentiometer.*

All of the H-ion concentration determinations which have been listed above were made using the colorimetric method. An additional series of experiments was run in which the H-ion concentrations were determined by both the colorimetric method and the electrometric method for the sake of comparison. A

Table XI.

Showing pH at Start and End of Experiments.

pH at Start of Experiment.	pH at End of Experiment.
5.0	5.8 to 6.2
5.2	6.4 to 6.6
5.4	6.8 to 7.2
5.6	7.2
5.8	7.4
6.0	7.4
6.2	7.6
6.4	7.6
6.6	7.6
6.8	7.6
7.0	7.8
7.2	7.8
7.4	7.8

Youden hydrogen ion concentration apparatus, manufactured by the W. M. Welch Co. of Chicago, was used in making the electrometric determinations. The H-ion concentrations determined with the potentiometer were consistently lower than those made using the color indicators, showing a difference in pH of .15 to .26 with a mean difference of .21. The reactions of *Paramecium*

Table XII.

Showing the Percentage Reactions of *Paramecia* to Hydrogen Ion Concentrations of pH 5.0–7.6 Made with Nitric Acid in Chicago Tap-Water and the Difference in Hydrogen Ion Concentrations when Measured with Both Color Indicators and Potentiometer.

pH Color.	pH Potentiometer.	Difference.	Reactions.
5.0	4.97	0.21	56
5.2	5.00	0.20	73
5.4	5.16	0.24	81
5.6	5.38	0.22	81
5.8	5.62	0.18	79
6.0	5.79	0.21	79
6.2	5.96	0.24	78
6.4	6.21	0.19	77
6.6	6.38	0.22	71
6.8	6.65	0.15	74
7.0	6.79	0.21	78
7.2	7.03	0.17	77
7.4	7.20	0.20	73
7.6	7.36	0.24	66
7.8 (tap water)	7.55	0.25	31
7.7 (culture)	7.44	0.26	

were about the same as those in the other experiments. The following table will show the results. The figures listed under the column "pH Potentiometer" represent the average of three determinations. The figures under the column "Difference" represent the average difference of the three determinations. The figures under the column "Reactions" represent the average reactions of five observations at each H-ion concentration.

It should be stated that the above comparison does not affect the results of the other experiments. This comparison merely shows that the pH range is moved back approximately 0.21 when the H-ion concentration is determined with the potentiometer used in this case. The almost identical mean determinations (4.97 and 5.00) with the potentiometer indicates the difficulty mentioned before in determining H-ion concentrations of pH 5.0 and pH 5.2 with the color indicators.

Discussion.

Jennings ('06) says that "specimens in water that is decidedly alkaline collect even more readily in acids than do those in a neutral fluid." This was found to be the case in these experiments except that *Paramecia* from slightly acid and neutral cultures did not give positive responses according to the method used in reckoning positive responses. Greely ('04) states that only *Paramecia* from acid cultures react positively to acids. This does not agree with Jennings. There is nothing in these experiments which is in agreement with this statement; in fact I have found the exact opposite to be true.

Child and Deviney ('26), Pruthi ('27) and many others have mentioned the different stages that occur in the life history of a protozoan culture. Four distinct stages were noticed in the cultures of *Paramecia* used in these experiments. The first stage was not very long in duration except in cases when Erlenmeyer flasks were used, and the H-ion concentration was high (pH 6.8–7.0). The second stage was marked by a steady fall in H-ion concentration. In the third stage the H-ion concentration was low and remained fairly constant. In this stage the *Paramecia* were scattered throughout the culture. Most of the *Paramecia* used in these experiments were in this stage. The last stage was marked by the *Paramecia* gathering in the bottom of the culture and dying out. *Paramecia* from cultures in the last stage were never used in the experiments because it seemed that the toxic substances in such a culture, as well as the general physiological state of the *Paramecia*, might prevent normal reactions.

An examination of all of the data shows that *Paramecium* (from cultures with a low H-ion concentration) react positively to all H-ion concentrations higher than that of their culture to a concentration of pH 5.0 inclusive. This limit for positive reactions is the same as Crane ('21) found to be the highest H-ion concentration in which *Paramecium* can live 24 hours. I have checked this observation and have obtained essentially the same results.

Close observations demonstrate rather clearly that *Paramecia* give the same response upon coming into contact with strong acids (more acid than pH 5.0) and upon coming into contact with their old culture medium after they are already in the acid solution (less acid than pH 5.0). In both cases they give the avoiding reaction.

Dr. L. G. Barth in his Doctor's Dissertation,[1] "The Effect of Acids and Alkalies on the Viscosity of Protoplasm," has found in unfertilized *Arbacia* eggs that acids if they are able to penetrate coagulate at pH 5.0. This suggests a reason why *Paramecia* are not able to live in concentrations of acids lower than pH 5.0.

Incidentally some observations were made on the toxicity of acid solutions. Pruthi ('27) observed that animals from a culture with a concentration of pH 7.8 placed in a hydrochloric acid solution with a concentration of pH 6.0 died within half an hour. Throughout these experiments it was observed that the organisms were in good condition after being in even stronger acids for considerable periods. Some *Paramecia* from a culture with a concentration of pH 7.8 were stirred into a citric acid solution with a concentration of pH 5.7. At the end of a half hour and an hour and a half some were placed back in culture media. In each case the animals seemed to suffer no ill effects and reproduced in an apparently normal manner. Again some *Paramecia*

[1] Placed in the University of Chicago Library in 1929. To be published later.

were placed in a hydrochloric acid solution with a concentration of pH 6.0 and left. Three days later the H-ion concentration had fallen to pH 6.2 and the animals were in normal condition. Pruthi also observed that *Paramecia* reproduce more rapidly in their own culture medium than in tap-water of the same H-ion concentration and suggests that this may be due to a greater amount of carbonates in the culture medium. To the writer of this paper it seems that a much more definite factor is food, as rate of division is closely correlated to amounts of food available.

Jennings' ('06) statement that "*Paramecia* collect in all weakly acid solutions, no matter what acid substance is present," was confirmed in these experiments. In the cases of pyrogallic acid and tannic acid no positive responses were obtained at the H-ion concentration used. To make solutions of these two acids with a pH range of 4.8–5.8, almost saturated solutions were necessary. It seems that some other factor besides the H-ion concentration is responsible for the great toxicity of these two acids. However, it was interesting to note that *Paramecia* reacted to weaker solutions of these two acids. In each case when pyrogallic or tannic acid was used the *Paramecia* gathered in the neck between the two drops and died there. This bears out another statement by Jennings to the effect that there is no relationship between the attracting or repelling power of acids and their injurious effects. Since it was apparent that all acids elicited the same responses, unless they were toxic in the pH range used, the three inorganic acids, hydrochloric, sulphuric and nitric acids, were used in most of the experiments in order that a more concentrated study might be made.

It is well known that carbon dioxide plays an important part in the behavior of *Paramecia* in natural conditions. The work of Hyman on Planaria ('25) made it appear that *Paramecia*, in reacting to acidified water, might be reacting to the carbon dioxide liberated from the carbonates instead of to free hydrogen ions. The series of experiments using carbonate-free water were run with the aim of finding out something in this connection. Although the writer realizes that many biologists today feel that too much significance has been ascribed to H-ion concentration "per se" in biological reactions, the results of the experiments using carbonate-free water give a strong indication that *Paramecia*, when reacting to weakly acid solutions, react to the free hydrogen ions. This idea does not seem to be out of line with the natural conditions that cause *Paramecia* to gather in clumps where carbon dioxide is present. The carbon dioxide in the cultures where *Paramecia* gather in clumps is in the form of carbonic acid.

Jennings ('06) has noted that all alkalies, save the alums, have a strong repellent effect on *Paramecia*. The observations made in these experiments that *Paramecia* will not react positively to a H-ion concentration lower than that of their culture tend to support this conclusion. The repellent effect of alkalies on *Paramecia* seems to help explain the fact that *Paramecia*, in cultures which have become quite alkaline (so far as is known the change in H-ion concentration in cultures cannot be regulated by the *Paramecia*), react to solutions with a H-ion concentration higher than that of their cultures.

Loeb ('18) based his conclusion that *Paramecia* are indifferent to acid solutions on Barratt's experiments using Pfeffer's capillary tubes. In repeating Barratt's work it seemed evident that the size of the openings in the tubes is the factor which explains the indifferent reactions. *Paramecia* cannot enter the small tubes without striking the edges. They can and will enter larger tubes containing weakly acid solutions.

The nature of the response of *Paramecia* to acid solutions received some consideration also. Jennings ('99) believed that the aggregations resulted from trap action, *i.e.* from a series of negative reactions to solutions of low acidity when the animals were in acid, rather than from a true positive chemotropism. Garrey ('02) working with *Chilomonas* concluded that the organisms showed positive chemotropism to acetic, lactic, and butyric acids, but that aggregations in inorganic acids resulted from trap action or "chemokinesis" as he called it. His conclusion was made possible by the manner of orientation and slow movement of the organisms he studied. The rapid movement of *Paramecia* through diffusion areas prevents accurate conclusions as to the nature of the responses to acids, so it was not possible to distinguish between the two types of reaction as observed by Garrey. It seemed evident, in these experiments, that the *Paramecia* reached the acid region by trial or random movements, and that once within the acid trap action occurred. Crossing back and forth between drops which had stood a long time was often noticed but in such cases the indicators would usually show that complete diffusion had taken place throughout both drops.

In the experiments when carbonate-free water or distilled water was used NaOH was added to lower the H-ion concentration in each case to pH 7.8. In all of these experiments the *Paramecia* tended to gather in clumps in a much more striking way than they did in any of the other experiments. In some cases the clumping occurred in the neck between the two drops, making it necessary to discard such experiments. In these experiments hydrochloric, sulphuric, nitric and carbonic acids were added to the water, altered with NaOH to produce the desired H-ion concentrations. As a result NaCl, Na_2SO_4, $NaNO_3$ or Na_2CO_3 was formed in the solutions. Jennings ('06) has listed NaCl, $NaNO_3$ and Na_2CO_3 as being very repellent to *Paramecia*. He further states that when a repellent substance, as NaCl, is mixed with an acid and a drop of the mixture is placed on a slide of *Paramecia* the *Paramecia* gather in a ring about the drop. In these experiments it seems that the attracting power of the H-ions is greater than the repellent power of the salts formed.

One of the most puzzling features of the whole series of experiments was the failure of the *Paramecia* to react positively to Whitman well water. Although analyses of Whitman well water and Hull well water were obtained for reference, no possible cause for the difference in eliciting positive responses can be given at this time. The *Paramecia* seem to serve as very delicate indicators in this connection, and the fact that *Paramecia* do not react positively to Whitman well water may be of importance in future experimentation with this water.

Summary.

1. The reactions of *Paramecium caudatum* to solutions of known hydrogen ion concentration were studied.

2. The colorimetric method of determining hydrogen ion

concentration was used but was checked by a series determined both colorimetrically and by Youden's potentiometer.

3. The acids used were: hydrochloric, nitric, sulphuric, carbonic, formic, acetic, citric, picric, pyrogallic and tannic.

4. The waters used were: Chicago tap-water, Whitman well water, Hull well water, Crawfordsville (Ind.) tap-water, distilled water, Chicago tap-water made carbonate-free, Whitman well water made carbonate-free, and Hull well water made carbonate-free.

5. In these experiments only *Paramecia* from alkaline cultures responded positively to acidified solutions.

6. *Paramecia* from alkaline cultures react positively to all H-ion concentrations higher than that of their culture to pH 5.0 (4.83 potentiometer reading) inclusive, when the H-ion concentration is produced by the inorganic acids—hydrochloric, sulphuric and nitric acids.

7. Organic acids, such as acetic, formic, carbonic and citric, which are not toxic in this pH range elicit responses similar to those caused by the inorganic acids.

8. Pyrogallic and tannic acids are toxic to *Paramecia* in solutions with concentrations of pH 4.8 through pH 5.8 but the organisms enter these waters and die as a result.

9. *Paramecia* react to acidified water from which all carbonates have been previously removed. This is an indication that *Paramecia* react to the hydrogen ion "per se" and not to carbon dioxide.

10. *Paramecia* are better able to live in solutions of high H-ion concentration than many have supposed.

11. Sulphonphthalein indicators, when used in no stronger concentration than necessary for pH determinations, are not harmful to *Paramecia.*

12. *Paramecia* will not react positively to weakly acid solutions when the temperature is below 21 degrees C.

13. No new evidence was obtained as to the nature of the mechanics of the reaction of *Paramecia* to acids.

14. *Paramecia* gather in clumps when they react to acidified solutions containing NaOH.

15. In one of the four types of water tested acidified water did

not elicit positive responses from *Paramecia*. No reason is offered for this exception.

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